

A REVIEW ON USE OF NITRIFICATION INDICATORS FOR INCREASING NITROGEN USE EFFICIENCY

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ABSTRACT

The rapid conversion of NH_4^+ to NO_3^- in soil results in the inefficient use of both soil N and applied N fertilizer. Nitrification inhibitors (NIs) are chemicals that inhibit or retard oxidation of ammonium to nitrate N. Use of NIs increases the efficiency of N-fertilizers, because nitrates formed on the oxidation of ammonium are easily lost by leaching under upland and denitrification under submerged soil conditions.

A large number of chemicals are known to have nitrification inhibiting properties such as N-Serve, DCD, ATS etc. but there are also some non-synthetic ecofriendly NIs which have inhibiting properties viz., Neem cake, Tea waste etc. A newly developed non synthetic NI that has been becoming popular is the Biological Nitrification Inhibititon (BNI). BNI capacity is widespread among the crop and pastures. A tropical pasture grass *Brachiaria humidicola* showed that BNIs can provide sufficient inhibitory activity soil nitrification and nitrous oxide emissions

In theory, reducing nitrification under conditions where there is a high risk of N loss from NO_3^- leaching or denitrification, improved nitrogen use efficiency (NUE) .By applying NIs we can not only inhibit the nitrification process but can also increases the NUE, as well as can prevent different losses of nitrogen.

KEYWORDS: Nitrification Inhibitors, Biological Nitrification Inhibitors, Nutrient Use Efficiency

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INTRODUCTION

Nitrification, a microbial process, is a key component and integral part of the soil nitrogen (N) cycle. It is the biological oxidation of ammonia (NH_3) to nitrate (NO_3^-) and is carried out by two groups of chemolithotrophic bacteria (*Nitrosomonas* spp. and *Nitrobacter* spp.), which are ubiquitous on earth (Norton et al., 2002). In agricultural systems, rapid and unchecked nitrification, however, results in inefficient N use, N leakage, and environmental pollution (Subbarao et al., 2009). The NO_3^- formed, is highly susceptible to losses from the root zone by leaching and/or denitrification (Subbarao et al., 2006). Loss of N from the root zone has large economic implications, just as fertilizer losses alone, besides the unknown cost of environmental consequences such as nitrate (NO_3^-) pollution of ground water, eutrophication of surface waters, and atmosphere pollution. Management of nitrification by the application of chemical inhibitors is a proven strategy to improve N recovery, agronomic N use efficiency (NUE), and for limiting environmental pollution (Sahrawat and Keeney, 1985; Subbarao et al., 2006;

Prasad, 2009). Several synthetic chemicals capable of inhibition of urea hydrolysis or nitrification in soils have been evaluated. Examples include N-serve (nitrapyrin), dicyandiamide (DCD), AM (2-amino-4 chloro-6 methyle pyrimidine), sodium chlorate, sodium azide, and benzene hexachloride ($C_6H_6C_{16}$). Many of these products, however, have been restricted to the experimental stage because of the high cost, limited availability, and adverse influence on beneficial soil microorganisms and, above all, poor extension and promotional activities for taking the technology to the farmers. Plant based nitrification inhibitors, which are eco-friendly and biodegradable, therefore hold considerable promise. Indeed, suppression of soil nitrification has been observed in some natural ecosystems (natural nitrification inhibition). It aims conservation of soil N and improved N status through development of low NO_3^- – ecosystems (Lata *et al.*, 2004; Subbarao *et al.*, 2006). Therefore use of nitrification inhibitors is one of the option for increasing the nitrogen use efficiency (NUE's).

Why to use Nitrification Inhibitors ?

- When we apply nitrogenous fertilizers, ammonium forms of N have quickly convert into nitrate forms. Inhibitors reduce this risk.
- To reduce different nitrogen losses and to increase the Nitrogen Use Efficiency (NUE)
- Better exploitation of N through adapted delivery of nitrogen
- Secure supply of nitrogen irrespective of the weather
- Work and cost saving through fewer applications of fertilizer

There are different types of nitrification inhibitors ,broadly they are classified in to two groups : Specific and non-specific nitrification inhibitors. Specific Nitrification Inhibitors such as N- Serve, AM etc., and Non- Specific Nitrification Inhibitors Such as Herbicides, Insecticides etc.,A new group of nitrification inhibitors is Biological Nitrification Inhibitors which had been discussed below.

BIOLOGICAL NITRIFICATION INHIBITORS (BNI)

The ability to produce and release nitrification inhibitors from plant roots to suppress soil nitrifier activity is termed ‘biological nitrification inhibition’ (Figure 1). Certain plants can suppress soil nitrification by releasing inhibitors from roots, a phenomenon termed ‘biological nitrification inhibition’ (BNI) .In ecosystems with large amounts of BNI (e.g. brachialactone), such as in *Brachiaria* grasses, the flow of N from NH_4^+ to NO_3^- via NO_2^- is restricted, and it is NH_4^+ and microbial N rather than NO_3^- that accumulates in the soil. In systems with little or no BNI, such as modern agricultural systems, nitrification occurs rapidly, leaving little time for plant roots to absorb NO_3^- ; thus NO_3^- is lost from the system through denitrification and leaching .BNI function has the potential to improve agronomic NUE (Subbarao *et.al.*, 2012, 2013b).

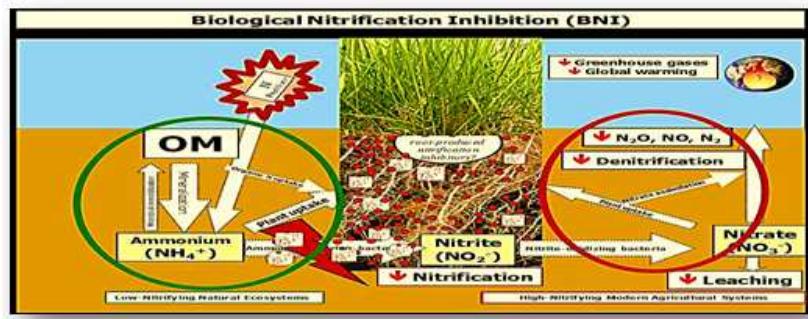


Figure 1: Schematic Representation of the Biological Nitrification Inhibition (BNI) Interfaces with the N Cycle

How Biological Nitrification Inhibitors (BNI) Works?

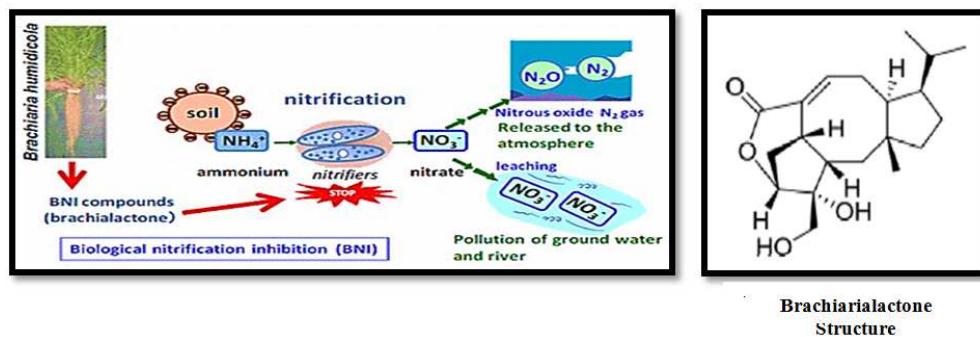


Figure 2

In the above figure, a chemical brachialactone has been released by a pasture grass *Brachiaria humidicola* that stops the activity of the nitrifiers for functioning and hence the nitrification rate goes down and the applied fertilisers remain in the soil for a longer period of time. Also, the brachialactone chemical structure has been shown.

Different case studies are mentioned below which will support the following review study are given below:

Table 1: Plant Product/ byproduct, Derivatives used as Nitrification Inhibitors (NI) and the Relative Efficiency for Promoting N use Efficiency

Nitrification Inhibitors	Source	Increase in NUE (%) (Compared to no NI)	References
Neem cake/ Neem oil emulsion	<i>Azadirachta indica</i> Adr. Juss.	20–25	Bains <i>et.al.</i> , (1971); Prasad <i>et.al.</i> , (1999)
Nimin	<i>A. indica</i> oil	25–30	Vyas <i>et.al.</i> , (1981)
<i>Citrullus</i> cake.	<i>Citrullus colocynthis</i> (L.) Schrad	20–25	Jain <i>et.al.</i> , (1980)
Karanj cake	<i>Pongamia glabra</i> Vent.	20–25	Prasad <i>et.al.</i> , (1971)
Karanjin	<i>P. glabra</i> seeds	20–25	Sahrawat (1981a; b)
Mint essential oil.	<i>Mentha spicata</i> L	30–35	Patra <i>et.al.</i> , (2001; 2002; 2009)
Byproducts	Medicinal and aromatic plants	30–35	Kiran and Patra (2003)
Byproducts	Natural essential oils	20–25	Kiran <i>et.al.</i> , (2003)

In table 1, different plant product/ byproduct, derivatives used as nitrification inhibitors (NI) and their relative efficiency for promoting N use efficiency were shown. Here, Mint essential oil and by products of medicinal and aromatic plants has the highest nitrogen use efficiency (NUE) i.e., 30-35% as compared to others. (Upadhyay *et.al.*,2011)

Table 2: Relative Efficiency of Prilled Urea and Neem-Urea Products for Rice

Treatment Productive	Tillers per hill (<i>n</i>)	Panicle length (cm)	Panicle weight (g)	1000-grain weight (g)	Grain (t/ ha)	Straw (t /ha)
Control	7.0	23.8	1.91	20.2	3.4	6.7
Prilled urea	7.6	25.4	2.15	21.3	4.0	7.7
Pusa neem golden urea	7.7	26.2	2.19	21.6	4.3	8.3
Pusa neem microemulsion urea	8.0	27.3	2.11	21.6	4.2	7.9
Neem cake coated urea	8.2	27.5	2.16	21.3	4.1	8.0
LSD(0.05)	0.045	0.84	0.125	0.10	0.43	0.58

These sources of N were applied at 120 kg N ha⁻¹

A field study made with rice at the Indian Agricultural Research Institute, New Delhi, showed that coating urea with neem oil, neem cake or neem oil microemulsion improved rice growth and resulted in more grain and straw than did commercial prilled urea. It also shows that coating of urea with neem oil emulsion, while imparting better physical properties to urea, has no adverse effects on urea as a fertilizer for rice; on the contrary it proves slightly better. (Prasad *et. al.*, 1999)

Bundy and Bremner (1973): Studied the inhibition of Nitrification in soils. Mainly 3 soils: Harps soil, Webster Soil and Storden soil were studied. The properties of these soils are given below based on which the different effect of nitrification inhibitors in Table no 5, 6 and 7 with Ammonium N and Urea N at various temperatures are given below.

Table 3

Soil Type	pH	CEC	Organic C (%)	Total N (%)	Sand (%)	Clay(%)
Harper	7.6	38	5.02	0.405	24	34
Webster	7.3	39	3.54	0.272	29	33
Storden	7.2	18	1.65	0.135	55	21

Table 4: Effects of Nitrification Inhibitors on Nitrification of Ammonium N Added To Soils (30 °C)

Inhibitor	Harps Soil (%)	Webster Soil (%)	Storden Soil (%)	Average (%)
2-chloro-6-(trichloromethyl) -pyridine	69(91)	80	97	82
4-Amino-1,2,4-triazole	66(82)	76	92	78
Sodium azide	58(64)	72	94	75
Potassium azide	56(63)	72	94	74
2,4-Diamino-6-trichloromethyl-a-triazine	36(79)	63	97	65
Dicyandiamide	20(46)	64	76	53

Table 4: Contd.,				
3-Chloroacetaanilide	16(58)	23	93	44
1-Amidino-2-thiourea	4(62)	43	74	41
3-Mercapto-1,2,4-triazole	6	26	64	32
2-Amino-4-chloro—6 –methyl-pyrimidine	0(30)	22	69	31
Sulfathiazole	0(17)	23	39	20
Sodium diethyl dithio carbamate	0	19	18	12

(Figures in parentheses are results obtained when 5g of Harps soil mixed with 5g sand)

Table 3 shows the results obtained with 12 of the 24 inhibitors studied in the comparisons of the effects of 10 ppm of these inhibitors on nitrification in the three soils incubated at 30 degree C for 14 days after treatment with 200 ppm of N as ammonium sulphate .The data showed that, with the soil used the average effectiveness of the most potent nitrification inhibitors decreased in the order: 2 chloro-6-trichloro methyl pyridine (N-Serve) > 4-amino-1,2,4-triazole (ATC)> Sodium or potassium azide> 2,4- diamino-6 trichloro-s- triazine > dicyandiamide> 3 chloro-acetanilide > 1 amidino-2 – thiourea >3 mercapto- 1,2,4 triazole or 2 amino-4 chloro-6 methyl pyrimidine (AM) > sulfathiazole (ST) >sodium diethyldithiocarbamate.

Table 5: Effects of Various Nitrification Inhibitors on Nitrification of Ammonium N Added to Soils (15⁰ C)

Inhibitor	Harps Soil (%)	Webster Soil(%)
2-chloro-6-(trichloromethyl) – pyridine	87	89
4-Amino-1,2,4-triazole	95	92
Sodium azide	62	88
Potassium azide	61	87
2,4-Diamino-6-trichloromethyl-a- triazine	46	80
Dicyandiamide	69	71
3-Chloroacetaanilide	65	54
1-Amidino-2-thiourea	67	46
3-Mercapto-1,2,4-triazole	55	57
2-Amino-4-chloro—6 –methyl-pyrimidine	32	50
Sulfathiazole	14	25
Sodium diethyl dithio carbamate	24	36

Table 4, shows the results of experiments to determine the effects of various nitrification inhibitors on nitrification of ammonium in soils at 15⁰C. The following table has the difference with table 5 is that incubation was performed at 15⁰C instead of 30⁰C and for 28 days instead of 14 days .It is evident from comparison of the data in table 5 and table 6 that all

the nitrification inhibitors studied were more effective at 15⁰C than 30⁰ C and that temperature also influenced the relative effectiveness of these compounds. For e.g., N-Serve was more effective than ATC at 30⁰C but was less effective than ATC at 15⁰C.

Table 6: Effects of Various Nitrification Inhibitors on Nitrification of Urea N Added to Soils (30⁰ C)

Inhibitor	Harps Soil(%)	Webster Soil(%)
2-chloro-6-(trichloromethyl) -pyridine	74	94
4-Amino-1,2,4-triazole	39	60
Sodium azide	34	49
Potassium azide	35	54
2,4-Diamino-6-trichloromethyl-a-triazine	21	69
Dicyandiamide	0	27
3-Chloroacetaanilide	2	17
1-Amidino-2-thiourea	0	17
3-Mercapto-1,2,4-triazole	2	20
2-Amino-4-chloro—6 —methyl-pyrimidine	0	29
Sulfathiazole	0	7
Sodium diethyl dithio carbamate	0	0

Table 5, shows the results of a study of the effects of various nitrification inhibitors on nitrification in soils treated with urea in Harps and Webster soils. Comparisons of data in table 5 and table 7 shows that, although some of the inhibitors (e.g., N-Serve) were more effective with urea than with ammonium sulfate. Hence, with various types of fertilizers effectiveness varies. So, to maximize the effectiveness compatible nitrification inhibitors to be used for better performance as well increasing the nitrogen use efficiency (NUE) of the crop.

Table 7: Effect of Nitrification Inhibitors on Rice Production

Treatments	Grain (g/pot)	Straw (g/pot)	Total (g/pot)
Control	26.60	37.50	67.1
Urea	49.40	53.90	103.3
Urea + N serve	62.10	64.30	126.4
Urea + 0.02% neem cake	52.00	56.20	108.2
Urea + 0.04% neem cake	63.70	66.60	130.3
Urea + 0.02% tea waste	50.70	59.20	109.9
Urea + 0.04% tea waste	51.30	58.00	109.3
L.S.D0.05	1.22	2.75	-
L.S.D 0.01	1.72	3.85	-
Ammonium Sulphate (AS)	51.20	55.10	106.3

Table 7: Contd.,			
AS+ N serve	59.80	55.0	115.7
AS + 0.02% neem cake	59.00	62.60	121.6
AS + 0.04% neem cake	64.60	68.90	123.5
AS + 0.02% tea waste	50.40	57.80	108.2
AS + 0.04% tea waste	49.90	56.20	106.1
L.S.D0.05	1.58	2.53	-
L.S.D 0.01	2.22	3.62	-

In table 6, different effects of Nitrification Inhibitors on rice production were shown. The following treatments were selected: Control, Urea, Urea + N serve, Urea + 0.02% Neem cake, Urea + 0.04% Neem cake, Urea + 0.02% tea Waste, Urea+ 0.04% tea waste. Out of which Urea + 0.04% Neem Cake shows the best result which increase both grain and straw yield followed by Urea + N –serve. Similar kinds of results were found when in place of urea ammonium sulphate was used in the experiment. (El-Galil *et.al.*, 1999)

Table 8: Nitrogen Content in Rice Plants and its Uptake as Affected by N-source and Nitrification Inhibitors

Treatments	N-Content (%)		N-Uptake(Mg/Pot)		Total
	Grain	Straw	Grain	Straw	
Control	0.79	0.57	234	214	448
Urea	0.84	0.59	415	318	733
Urea+N serve	0.87	0.63	540	405	945
Urea+0.02% neem cake	0.84	0.59	437	332	769
Urea + 0.04% neem cake	0.85	0.63	541	420	961
Urea +0.02% tea waste	0.79	0.62	401	367	768
Urea + 0.04% tea waste	0.81	0.61	416	354	770
Ammonium Sulphate	0.81	0.58	415	320	735
AS+ N-serve	0.83	0.60	496	335	831
AS+ 0.02% neem cake	0.79	0.61	466	382	848
AS+ 0.04% neem cake	0.84	0.59	543	407	950
AS + 0.02% tea waste	0.79	0.50	398	341	739
AS+0.04% tea waste	0.78	0.64	389	360	749

AS: Ammonium Sulphate

In table 7, nitrogen content in rice plants and its uptake as affected by N-source and nitrification inhibitors. In this experiment treatments were Control, Urea, Urea + N serve, Urea + 0.02% Neem cake, Urea + 0.04% Neem cake, Urea +

0.02% tea Waste, Urea+ 0.04% tea waste, AS, AS+ N serve, AS + 0.02% Neem cake, AS + 0.04% Neem cake, AS + 0.02% tea Waste, AS+ 0.04% tea waste .The result revealed that along with fertilisers viz., Urea or Ammonium Sulphate if 0.04% Neem cake applied than N content and N- uptake in both grain and straw increased. This is due to the fact that as it slows down the Nitrification process that makes the availability of the nutrients for a longer period. (El-Galil *et.al.*, 1999)

Table 9: N-Recovery and Utilization Efficiency (%) as Affected by Nitrification Inhibitors

Treatments	N-Recovery mg/pot	Utilization Efficiency (%)
Urea	285	47.5
Urea+N serve	497	82.8
Urea+0.02% neem cake	321	53.5
Urea + 0.04% neem cake	513	85.5
Urea +0.02% tea waste	320	53.3
Urea + 0.04% tea waste	322	53.7
Ammonium Sulphate	287	47.8
AS+ N-serve	383	63.8
AS+ 0.02% neem cake	400	66.7
AS+ 0.04% neem cake	502	83.7
AS + 0.02% tea waste	291	48.5
AS+0.04% tea waste	301	50.2

In table 8, N-recovery and utilization efficiency (%) as affected by nitrification inhibitors in rice plants were shown. In this experiment treatments were Control, Urea, Urea + N serve, Urea + 0.02% Neem cake, Urea + 0.04% Neem cake, Urea + 0.02% tea Waste, Urea+ 0.04% tea waste, AS, AS+ N serve, AS + 0.02% Neem cake, AS + 0.04% Neem cake, AS + 0.02% tea Waste, AS+ 0.04% tea waste .The result showed that along with Urea or Ammonium Sulphate if 0.04% Neem cake applied than N recovery and Utilisation efficiency increased followed by N- Serve combination treatments. This is due to the higher use efficiency of the applied nutrients. (El-Galil *et.al.*, 1999)

Table 10: Effect of Nitrification Inhibitors on Rice Nutrients Content In Soil after Harvesting (Available Nutrients Mg/ Kg Soil)

Treatments	Inorganic N	P
Control	23.1	11.1
Urea	37.3	19.4
Urea+N serve	65.1	24.2
Urea+0.02% neem cake	43.1	21.1
Urea + 0.04% neem cake	55.9	23.6
Urea +0.02% tea waste	36.6	17.5
Urea + 0.04% tea waste	34.4	18.3
Ammonium Sulphate	33.2	20.2

Table 10: Contd.,		
AS+ N-serve	54.2	26.4
AS+ 0.02% neem cake	46.5	24.3
AS+ 0.04% neem cake	52.9	26.1
AS + 0.02% tea waste	30.2	18.5
AS+0.04% tea waste	36.3	18.4

In table 9, effect of Nitrification Inhibitors on rice nutrients content in soil after harvesting had sown. In this experiment treatments were Control, Urea, Urea + N serve, Urea + 0.02% Neem cake, Urea + 0.04% Neem cake, Urea + 0.02% tea Waste, Urea+ 0.04% tea waste, AS, AS+ N serve, AS + 0.02% Neem cake, AS + 0.04% Neem cake, AS + 0.02% tea Waste, AS+ 0.04% tea waste .Urea + N-Serve and AS + N – Serve gives high residual effect. Inorganic N, P and K content in the soil after harvest of rice were measured and found that in N- serve treatments the nutrients were highly found. (El-Galil *et.al.*, 1999)

ADVANTAGES AND DISADVANTAGES OF NITRIFICATION INHIBITORS

Advantages

Improved nitrogen fertilizer management has many environmental benefits such as:

- Reductions in N_2O emission.
- Increase in N use efficiency will reduce leaching of NO_3^- to ground water.
- Decreased the need for inorganic N fertilizers and emissions from fossil fuel associated with their manufacture.

Disadvantages

- The use of chemical inhibitors of N_2O emissions may leave unacceptable residues, and they may not be effective in certain types of soil.
- High cost, so not affordable by farmers.

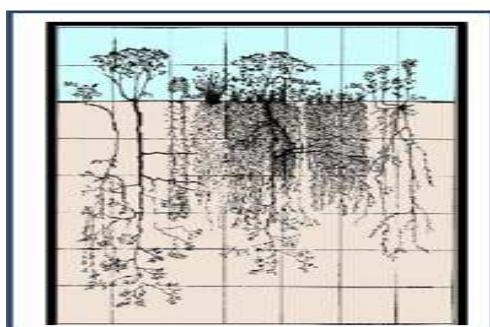


Figure 3

ON-GOING RESEARCH

Biological Nitrification Inhibitors

- Root exudates can inhibit soil conversion of ammonium
- to nitrate
- Useful with urea fertilizers
- Stops leaching in wet soils

(CIMMYT, Mexico)

GM technology-faster ammonium assimilation

- Alanine aminotransferase
- OSR:50%N required
- Now being transferred to rice, wheat, & maize

(Arcadia Biosciences, CA)

FUTURE RESEARCH THRUST

- Need to study the nitrification inhibitors in diversified crops after application of different nitrogenous fertilizers.
- More focus and study required by the researchers/ scientists in Biological Nitrification Inhibitors (BNI).
- Need to identify the constraints in adoption of nitrification inhibitors by the farmers for further refinement and progress of the technology.
- Use of genetically modified rhizosphere which can secrete more BNI that can help reducing N-application.
- Utilisation of BNI function for the development of breeding materials and application to cropping systems

SUMMARY AND CONCLUSIONS

It is very difficult to protect applied nitrogenous fertilizers from undergoing different losses from the system. Through nitrification inhibitors a major part of nitrogen loss can be reduced. NUE increased due to usage of nitrification inhibitors cost effective production can be attained. Chemical inhibitors eg., N-Serve and DCD can be potentially used in this inhibition process. Biological nitrification inhibitors (BNI's) can be used to inhibit the nitrification process which is eco-friendly .Different Nitrification Inhibitors have different efficiencies in inhibiting the Nitrification process .Growth of the bacteria responsible for Nitrification (*Nitrosomonas*) is inhibited significantly by use of NIs. Grain yield, Straw yield, N-content (%) as well as N-uptake in rice plants can be increased through adequate use of Nitrification Inhibitors (NIs).

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